IOWA STATE UNIVERSITY
College of Agriculture and Life Sciences

Inactivation of chromosomal genes of the choline bacterial microcompartment through transformation of *Escherichia coli* 536

Tiffany Nicole Harris, Department of Microbiology, Iowa State University, Ames, IA
Dr. Thomas Bobik, Department of Biochemistry, Biophysics, and Molecular Biology, Iowa State University, Ames, IA

ABSTRACT

- Bacterial microcompartments are proteinaceous organelle structures (BMC) (1) (**Fig 1**)
- Choline degradation forming trimethylamine (TMA) is suggested to occur in BMCs
- Objectives of study were to:
  - Use PCR products to inactivate genes in the choline utilization *(cut)* operon using linear transformation (2)
  - Identify and verify successful deletion of genes
  - Test mutants for choline degradation
- High levels of false positives have made it difficult to isolate successfully transformed cells

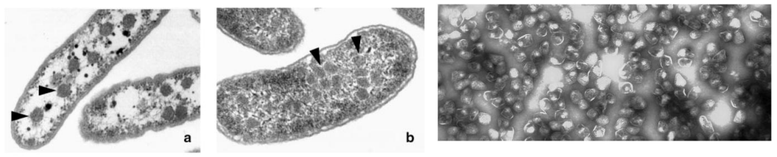

Fig 1. Visualization of BMCs. Electron microscope image of BMCs within cells (Left). Electron image of isolated BMCs (Right).
Image Credit: Cheng et al (3)

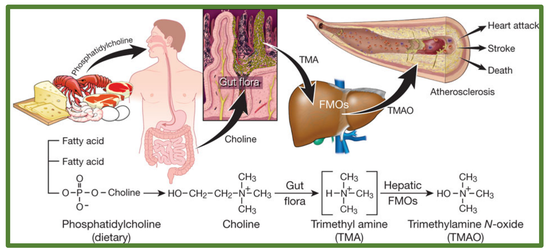

Fig 2. Association between TMA and cardiovascular disease. Image Credit: Wang et al (4)

INTRODUCTION

- BMCs allow a controlled space for enzymatic activity such as the breakdown of choline forming TMA and acetaldehyde reducing the toxicity of the aldehyde (1,3,6)
- Higher concentrations of TMA in plasma which is produced by bacteria in the human gut is correlated with a higher risk of cardiovascular disease (**Fig 2**) (4)
- Understanding how TMA is produced can lead to development of therapies for cardiovascular disease

MATERIALS AND METHODS

Preparing DNA for transformation by PCR (2)
- PCR with primer homologs and template strain with pKD4 which codes for kanamycin resistance
- Purification of amplified DNA by gel electrophoresis

Transformation using PCR products (2)
- Wanner and Datsenko protocol using pKD46 with λ red recombinase system (**Fig 3**)

Verification of successful gene deletion
- Mutants identified and colony PCR performed with flanking primers
- Gel electrophoresis of products for visualization

Testing for choline degradation
- Verified mutants by plating on choline MacConkey

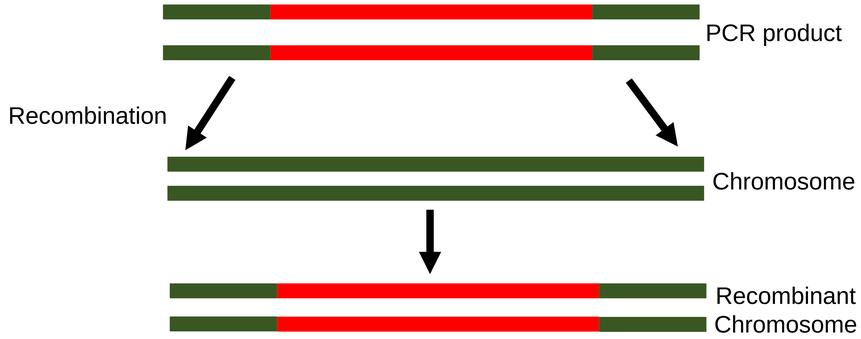

Fig 3. Linear transformation by the λ red recombinase system. PCR primers are made with DNA sequence homology of the end regions of the chromosomal target gene and undergo PCR with a kanamycin resistance gene sequence to produce a PCR product with a kanamycin resistance gene flanked by the chromosomal target gene end regions. The PCR product is electroporated into cells and undergoes recombination replacing the chromosomal target gene with the kanamycin resistance gene. Selection for successful transformants is done by plating on kanamycin medium which should only allow growth of those cells which received the resistance gene. (Chromosomal DNA is green, kanamycin resistance gene is red)

RESULTS

Attempts to delete ten genes associated with the choline BMC resulted in the production of numerous false positives making it difficult to identify successfully transformed mutants. Because of this no information regarding the effects of gene inactivation on choline degradation has been found.

DISCUSSION

Efforts to delete genes involved in choline degradation using PCR products has thus far been unsuccessful. Following transformation, verification that the proper genes had been inactivated and replaced by a gene for kanamycin resistance was conducted. Although PCR indicated a few genes were successfully deleted, future tests to isolate pure cultures resulted in no growth on kanamycin medium.
These results suggest that false positives were indistinguishable from properly transformed colonies. The protocol for transformation was altered slightly so that cells would now obtain chloramphenicol resistance instead of kanamycin resistance. This method should produce fewer false positives. As of now, transformation using the new protocol is currently underway.
The exact reasons for the large number of false positives that have been obtained in this study are unknown; however it is possible that this strain of *E.coli* mutates spontaneously resulting in kanamycin resistant cells. Low levels of natural resistance may also contribute; allowing some cells to escape selection.

ACKNOWLEDGEMENTS

I would like to extend a special thanks to Dr. Bobik for allowing me to participate in this project. Additionally I would like to thank Chiranjit Chowdhury, a post-doctorate student in Dr. Bobik's lab, for his assistance during the project, as well as Taylor Herring, another undergraduate student who worked with me on this project.

REFERENCES

1. **Bobik TA, Lehman BP, Yeates TO.** 2015. Bacterial microcompartments: widespread prokaryotic organelles for isolation and optimization of metabolic pathways. Mol. Microbiol. **98**:193–207.
2. **Datsenko KA, Wanner BL.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. PNAS **97**: 6640-6645.
3. **Cheng S, Liu Y, Crowley CS, Yeates TO, Bobik TA.** 2008. Bacterial microcompartments: their properties and paradoxes. Bioessays **30**: 1084-1095.
4. **Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, Feldstein AE, Britt EB, Fu X, Chung Y, Wu Y, Schauer P, Smith JD, Allayee H, Tan WHW, DiDonato JA, Lusis AJ, Hazen SL.** 2011. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature **472**: 57-63.
5. **Brzuszkiewicz E, Bruggermann H, Liesgang H, Emmerth M, Olschlager T, Nagy G, Albermann K, Wagner C, Buchrieser C, Emody L, Gottschalk G, Hacker J, Dobrindt U.** 2006. How to become a uropathogen: comparative genomic analysis of extraintestinal pathogenic *Escherichia coli* strains. PNAS **103**:12879-12884.
6. **Craciun S, Balskus EP.** 2012. Microbial conversion of choline to trimethylamine requires glycyl radical enzyme. PNAS **109**:21307-21312.